

WHAT IS CLAIMED IS:

1. A process for producing a target fermentation product comprising:

(a) providing a fermentation medium containing a recombinantly-produced microorganism that over-produces a target fermentation product and contains a mutation which causes auxotrophic growth of the microorganism, wherein the auxotrophy within the microorganism does not compromise the ability of the microorganism to produce the target fermentation product; and

(b) supplying the medium with all substrates required for production of the fermentation product, a substrate for the target fermentation product, and a substrate complementing the auxotrophy.

2. A process according to claim 1 further comprising isolating the target fermentation product from the microorganism and/or the fermentation medium.

3. A process according to claim 1 wherein the substrate complementing the auxotrophy is provided to the fermentation medium at a concentration sufficient to maintain biomass growth at a defined growth rate, and the substrates required for production of the fermentation product are provided to the fermentation medium at concentrations that do not limit the ability of the microorganism to produce the fermentation product.

4. A process according to claim 3 wherein the ratio of the target fermentation substrate:substrate complementing the auxotrophy is from about 1:10,000,000 to about 1:10.

5. A process according to claim 4 wherein the ratio of the target fermentation substrate:substrate complementing the auxotrophy is from about 1:1,000,000 to about 1:100.

6. A process according to claim 1 wherein the target fermentation product is
5 selected from the group consisting of riboflavin, pantothenic acid, thiamin, folic acid, pyridoxine, and amino acids.

7. A process according to claim 6 wherein the target fermentation product is
riboflavin.

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8. A process according to claim 1 wherein the microorganism contains a polynucleotide sequence coding for one or more polypeptides with enzymatic activities for producing riboflavin and a transcription element which is not naturally associated with, but which is transcriptionally linked with the polynucleotide sequence in the microorganism.

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9. A process according to claim 8 wherein the polynucleotide sequence is linked with more than one transcription element.

10. A process according to claim 9 wherein the transcription element
20 comprises at least one promoter.

11. A process according to claim 8 wherein the microorganism contains more than one copy of the polynucleotide sequence.

12. A process according to claim 1 wherein the auxotrophy in the microorganism is selected from the group consisting of biotin, adenine, tryptophan, lysine, and combinations thereof.

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13. A process according to claim 12 wherein the auxotrophy in the microorganism is a biotin auxotrophy.

14. A process according to claim 12 wherein the auxotrophy in the
10 microorganism is an adenine auxotrophy.

15. A process according to claim 12 wherein the auxotrophy in the microorganism is a lysine auxotrophy.

15 16. A process according to claim 1 wherein the substrate complementing the auxotrophy is biotin and one of the substrates for the fermentation product is glucose.

17. A process according to claim 1 wherein the microorganism is a biotin auxotroph, the substrate complementing the auxotrophy is biotin, the substrate for the target
20 fermentation product is glucose, and the target fermentation product is riboflavin.

18. A process according to claim 1 wherein the microorganism is selected from the group consisting of *Escherichia*, *Bacillus*, *Cyanobacter*, *Streptomyces*, and *Corynebacteria*.

5 19. A process according to claim 18 wherein the microorganism is selected from the group consisting of *E. coli*, *B. subtilis*, *B. Amyloliquefaciens*, *B. lichiniiformis*, *C. glutamicum*, and *B. ammoniagenes*.

10 20. A process according to claim 19 wherein the microorganism is a *Bacillus subtilis* bacterium.

21. A process according to claim 20 wherein the microorganism is RB50::[pRF69]Bio⁻ or derivative thereof which retains the ability to over-produce riboflavin.

15 22. A process according to claim 21 wherein the microorganism is RB50::[pRF69]Bio⁻.

23. A process for decoupling production of a target fermentation product from biomass production in a fermentation medium comprising:

20 (a) providing a recombinantly produced microorganism that has been engineered to contain a polynucleotide sequence which encodes biosynthetic enzymes for a

target fermentation product, the maximal production of the target fermentation product being dependent on an unlimited supply of a target fermentation product substrate; and

(b) introducing an auxotrophy into the microorganism to control biomass production by limiting the concentration of a substrate complementing the auxotrophy
5 in the fermentation medium.

24. A process according to claim 23 wherein step (b) comprises introducing a polynucleotide comprising a deletion-insertion mutation into the genome of the microorganism to disrupt the microorganism's ability to produce a compound required for biomass production.

10 25. A process according to claim 24 wherein the polynucleotide comprises deletion-insertion mutations within a *bioFDB* gene cassette.

26. A process according to claim 23 wherein the introducing step comprises
15 transforming the microorganism with a polynucleotide sequence comprising a *bioFDB* deletion-insertion mutation.

27. A process according to claim 26 comprising transforming the microorganism with a polynucleotide sequence comprising SEQ ID NO:1.

20 28. A process according to claim 27 further comprising selecting for transformation of the microorganism.

29. A process according to claim 28 wherein selecting for the transformation comprises selecting for antibiotic resistance.

30. A process according to claim 29 wherein selecting for the transformation
5 comprises selecting for neomycin resistance.

31. A process according to claim 23 wherein the target fermentation product is riboflavin.

10 32. A production microorganism made by the process of claim 23.

33. A polynucleotide sequence which is selected from the group consisting of SEQ ID NO:1 or an auxotrophy-introducing homolog of SEQ ID NO:1, and polynucleotide sequences containing insertions, deletions, and substitutions of SEQ ID NO:1, which retain the
15 ability to cause an auxotrophy in a host cell.

34. A polynucleotide sequence according to claim 33 consisting of SEQ ID NO:1 or a homolog thereof, which retains its ability to cause an auxotrophy in a host cell.

20 35. A polynucleotide sequence according to claim 34 consisting of SEQ ID NO:1.

36. A host cell transformed with a polynucleotide sequence comprising SEQ ID NO:1 or a homolog of SEQ ID NO:1 which retains its ability to cause an auxotrophy in the host cell, or a polynucleotide sequence containing insertions, deletions, and substitutions of SEQ ID NO:1 which retains the ability to cause an auxotrophy in the host cell.

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37. A host cell according to claim 36 which is transformed with SEQ ID NO:1 or a fragment thereof, which retains its ability to cause an auxotrophy in a host cell..

38. A host cell according to claim 37 transformed with SEQ ID NO:1.

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39. A host cell according to claim 38 wherein the host cell is a *B. subtilis* cell.

40. An riboflavin production microorganism RB50 containing multiple copies of the engineered *rib* operon pRF69 transformed with the polynucleotide sequence of SEQ ID NO:1.

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